J Physiol 593.1 (2015) pp 25–27

EDITORIAL

Ionotropic glutamate receptors: alive and kicking

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It has been about 25 years since a landmark cloning study identified the molecular identity of the first of 18 different genes that encode the ionotropic glutamate receptor (iGluR) family (Hollmann *et al.* 1989). Since that time, we have witnessed major advances in our understanding of the biology of iGluRs. Breakthroughs in genetics provided our first insights into the many roles iGluRs fulfil in behaviour and disease (Mulle *et al.* 1998), with advances in biochemistry identifying the myriad of protein partners that shuttle iGluRs into and out of synapses (Nicoll *et al.* 2006; Sheng & Kim, 2011). The last decade has been dominated by structural biology, which has offered an unprecedented glimpse into the working life of the iGluR at atomic resolution (Gouaux, 2004; Mayer & Armstrong, 2004). Each great advance has drawn more and more distinct scientific disciplines into the iGluR field, making it a challenge to keep up with the latest technological developments and biological advances.

With this in mind, a group of iGluR aficionados looked for ways to bring the iGluR community together to enhance trainee development and bring greater collaboration amongst the disciplines. The solution was to hold an annual iGluR Retreat on a university campus within reach by car to minimize cost. With so many iGluR researchers residing within the northeast quadrant of North America, the idea was met with great enthusiasm. The inaugural iGluR Retreat was held on the campus of Cornell University in August 2013 organized by Dr. Linda Nowak and Robert Oswald (see Fig. 1), with Dr Gabriela Popescu hosting the 2014 Retreat at SUNY Buffalo. This issue of *The Journal of Physiology* brings together seven timely review articles that capture some of the new ideas, discussions and debates that arose during these meetings.

Dr Sasha Sobolevsky (Sobolevsky, 2015) from Columbia University reviews the landmark paper he published with colleagues in the Gouaux lab describing the full-length structure of the GluA2–AMPA receptor (Sobolevsky *et al.* 2009). Up until that point, structure–function studies focused on examining the isolated extracellular domains of the iGluR, such as the ligand-binding pocket (LBD) and amino-terminal (NTD). Although these studies have been invaluable, they left researchers wondering how conformational events initiated in extracellular domains propagated to transmembrane pore helices. By elucidating its full length, Sobolevsky and colleagues re-affirmed the modular nature of the iGluR structure with its twofold or Y-shaped symmetry but they also pulled out a number of surprises. The most unexpected being the occurrence of two conformationally distinct subunits within the tetramer, a finding entirely unexpected given the homomeric nature of the protein. As explained in his review, this distinction is made possible by symmetry mismatch between the extracellular (i.e. LBD and NTD) and pore domains of each subunit. How this structural distinction contributes to channel gating is not clear though it may offer insight into earlier attempts to link subunit stoichiometry to functional behaviour (e.g. Robert *et al.* 2001; Bowie & Lange, 2002). The full-length GluA2 structure was solved in complex with a competitive antagonist so the pore region is in the closed, inactive conformation. However, Dr Sobolevsky has provided three movie downloads to accompany the manuscript that will help readers visualize how the iGluR may transition from one conformation to another following agonist binding.

The review from the Wollmuth lab (Gan *et al.* 2015) of Stony Brook University sets out a working hypothesis to explain how AMPARs are assembled, extending their earlier findings uncovering the role of transmembrane region 4 (Salussolia *et al.* 2011). In their proposed model, the NTD is responsible for the formation of a dimer pool, with transmembrane regions facilitating the formation and stability of the tetramer. The LBD is proposed to influence assembly through a process they refer to as 'domain swapping'. The authors make a compelling argument for the need to better understand the assembly process given that all plasticity mechanisms of glutamatergic synapses involve switches in subunit composition (Kauer & Malenka, 2007; Paoletti *et al.* 2013). Finally, they speculate on a new era of drugs designed to control the assembly process that, in doing so, fine-tune the output of the glutamatergic synapse.

Dr Jim Huettner from Washington University School of Medicine in St Louis

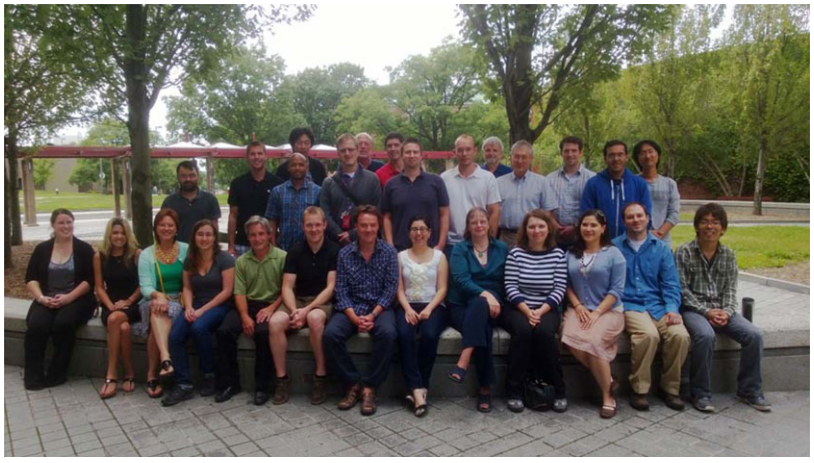

Figure 1. Photograph of some of the participants at the 2013 iGluR Retreat at Cornell University
Drs Linda Nowak (front row, 5th from right) and Robert Oswald (backrow, 4th from right) organized the 2013 Retreat with Dr Gabriela Popescu (front row, 3rd from left) organizing the 2014 Retreat in Buffalo, NY. Photo kindly provided by Dr Sasha Sobolevsky.

DOI: 10.1113/jphysiol.2014.284448

provides an interesting perspective on the structure and function of the ion channel pore – arguably the most elusive region of the iGluR (Huettner, 2015). Although the iGluR pore has similar architecture to the well-characterized potassium channel pore (Doyle *et al.* 1998; Panchenko *et al.* 2001), it continues to defy detailed structural and functional analysis. Firstly, unlike potassium channels, there is no accepted model of ion permeation for non-selective cation pores, such as iGluRs. And secondly, X-ray crystal structures capture the pore only in the closed conformation, making it difficult to extrapolate how it changes during activation. Despite these challenges, the Huettner lab has advanced our understanding of its architecture by arguing for a radial symmetry of the pore (Wilding *et al.* 2014). This latter point forms the central tenet of the review that structural differences between different iGluR subtypes accounts for their distinct pore properties.

Dr Jim Howe from Yale University provides a much-needed treatise on auxiliary proteins and how they shape the functional properties of AMPA- and KA-type iGluRs (Howe, 2015). Although this subject area has been reviewed extensively, the article is unique in that it describes how Type I TARPs (or transmembrane AMPAR regulatory proteins) and Neto1/Neto2 affect the *single* channel properties of AMPARs and KARs, respectively. TARPs shape the AMPAR response by increasing channel open probability and by promoting openings to the main activated state. Neto1 and Neto2 also increase the time KARs stay activated, in this case, by destabilizing desensitized states resulting in a slower decaying macroscopic response. Through insight from unitary recordings, Howe argues that auxiliary proteins greatly impact synaptic transmission by emphasizing the modal gating of iGluRs (Zhang *et al.* 2014) causing AMPARs and KARs to switch slowly between distinct gating states.

In their review, Drs Tim Green and Naushaba Nayeem from the University of Liverpool bring a structural perspective to the role of subunit interfaces in shaping iGluR behaviour (Green & Nayeem, 2015). Interfaces are located throughout the entire structure of the iGluR, from the extracellular NTDs and LBDs to the pore helices creating binding sites for numerous endogenous and exogenous factors. Not surprisingly then, conformational dynamics at subunit interfaces are linked to the gating processes of activation and desensitization, with the Green lab recently focusing on the LBD dimer interface of KARs which contains both anion and cation binding pockets (Nayeem *et al.* 2011). This same region of the LBD also contains binding sites for Ca^{2+} in Orphan GluD2 receptors (Naur *et al.* 2007) and Zn^{2+} in GluK3 KAR (Veran *et al.* 2012). The LBD ion binding sites of KARs are critical for function but it is still unclear how Ca^{2+} affects GluD2 receptors. Whether the occurrence of this cation binding pocket plays a role in establishing the unique non-ionotropic signalling of GluD2 receptors at glutamatergic synapse remains to be studied (Yuzaki, 2012).

The molecular and functional diversity of NMDA-type iGluRs is the focus of the article from the Johnson lab (Glasgow *et al.* 2015). Often considered the gatekeepers of plasticity mechanisms at glutamatergic synapses, NMDARs exhibit three key properties in this regard. Voltage-dependent channel block by Mg^{2+} keeps NMDARs essentially quiescent unless the neuron is depolarized by intense synaptic activity. At depolarized potentials, the NMDAR's tardy channel kinetics combined with its high Ca^{2+} permeability contributes to a sustained build-up of cytoplasmic Ca^{2+}. These events drive biochemical and genetic pathways that initiate and sustain long-lasting changes in synaptic efficacy. The authors bring out a much more nuanced view of NMDARs and explain how differences in subunit composition fine tune NMDAR activation properties and sensitivity to Mg^{2+} block. They point to two distinct structural regions of GluN2A–D subunits, with the NTD responsible for most subtype dependence in gating and ligand binding properties (Gielen *et al.* 2009) and a single amino acid residue, namely the GluN2 S/L site, being responsible for differences in ion permeation and block (Siegler Retchless *et al.* 2012; Clarke *et al.* 2013).

Finally, Dawe and colleagues re-examine the structural basis of activation emphasizing recent work on AMPA- and KA-type iGluRs (Dawe *et al.* 2015). Early models of iGluR activation pointed to the LBD as having a prominent role in this process, with the degree of domain closure determining the extent of channel activation (Madden, 2002). Since then, experiments on all iGluR families have identified pitfalls with this viewpoint. The authors argue that, at least for KARs, the LBD dimer interface may be more critical to consider since restricting its movements by covalent crosslinking disrupts transitions into full activation (Daniels *et al.* 2013). Furthermore, single-channel data show that the near-permanent occupancy of the cation binding pockets that line the LBD dimer interface keep GluK2 KARs in the main open state (Dawe *et al.* 2013), further suggesting that this region of the protein is a 'hotspot' for activation. AMPAR and NMDAR gating is not reliant on external ions in the same way as KARs (Bowie, 2010); however, curiously the LBD dimer interface possesses many of the same residues that constitute the cation binding pocket. Whether these similarities hint at a more general role for the LBD dimer interface in the activation of all iGluR families awaits future study.

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Additional information

Competing interests

None declared.